

## ***Sleeping Beauty* Vector System Moves Toward Human Trials in the United States**

**I**n a public review of a proposed clinical gene transfer trial, the National Institutes of Health Recombinant DNA Advisory Committee (NIH RAC) heard details in June 2008 of what is the first human trial in the United States utilizing the *Sleeping Beauty* (SB) transposon system. The trial is being proposed by Partow Kebriaei and Laurence Cooper at the University of Texas MD Anderson Cancer Center and collaborator Perry Hackett at the University of Minnesota. This trial will transfer genetically altered T cells into patients with CD19<sup>+</sup> B-lymphoid malignancies. The protocol is entitled “Adoptive Immunotherapy for CD19<sup>+</sup> B-Lymphoid Malignancies Using *Sleeping Beauty* Transposition to Express a CD19-specific Chimeric Antigen Receptor in Autologous *Ex Vivo* Expanded T Cells.”

The SB transposon system, derived from inactive sequences found in salmonid fish genomes, has been in development for therapeutic application for more than a decade. The SB system can be used to insert DNA into the genome via transposase-mediated DNA excision of a transgene from a recombinant plasmid containing internal repeats and insertion into TA sites of the genome.<sup>1</sup> The proposed protocol will utilize SB transposase 11, a hyperactive transposase expressed from a cytomegalovirus promoter, to excise a chimeric antigen receptor (CAR) expressed from the EF1- $\alpha$  promoter of a co-transfected second transposon plasmid for transduction of patient peripheral blood mononuclear cells. The CAR encompasses a CD19-specific murine single-chain variable fragment linked to the CD28 endodomain fused with the CD3- $\zeta$  cytoplasmic domain. Harvested peripheral blood mononuclear cells will be genetically modified by electroporation, and then, in a CAR-dependent manner, the T cells will be numerically expanded on irradiated K562 cells expressing CD19 and costimulatory molecules.<sup>2</sup> Study subjects will receive full myeloablative chemotherapy, a peripheral blood stem cell autologous transplantation, and monoclonal antibody to CD20 before infusion of autologous genetically modified T cells during the period of chemotherapy- and antibody-induced lymphopenia. The study seeks to determine the

feasibility, safety, and persistence of genetically modified T cells *in vivo* and also has several secondary objectives.

Data presented with the protocol demonstrated the ability to propagate the transfected T cells to clinically relevant numbers using the K562 cells as antigen-presenting stimulatory cells that are being made as clinical-grade material with support from National Heart, Lung, and Blood Institute Production Assistance for Cell Therapies program. These T cells express easily detectable (by immunoblot) levels of the CAR and function to lyse CD19-expressing target cells *in vitro*. Importantly, these cells do not express detectable levels of transposase beyond 28 days in culture. The technology is remarkable for the antigen-driven outgrowth of T cells with stable CAR expression using off-the-shelf reagents and for the lack of a requirement for any immunogenic drug-selection scheme.

One of several questions concerning the safety of using SB in human trials is the effect on the genome of expressing a transposase in patients' cells and the associated theoretical risk of insertional mutagenesis in these cells. There is a wealth of basic research addressing these issues in nonclinical studies but little direct human data that can be used to infer safety *in vivo*. Data suggesting that this system may be even safer than currently used  $\gamma$ -retroviruses (and lentivirus vectors) include the nearly random pattern of integration mediated by the SB transposase at TA sites (estimated to be about 200 million in the genome) and at sites distant from expressed genes and transcriptional units. Studies have shown the duration of expressed transposase activity to be short (e.g., only days *in vivo* in mice), and that there exists a potential autoregulatory mechanism that inhibits transposase activity. This autoregulatory mechanism seems to be mediated via the generation of an N-terminal dominant-negative peptide. No genomic instability has been demonstrated in limited human cell studies. Nonetheless, one recommendation of the RAC was for the investigators to provide an extended culture period of the modified T cells so as to evaluate further the possibility of clonal

expansion. In addition, although recognizing the limitations of currently available *in vivo* models, the RAC also suggested that further studies be conducted to determine the potential for insertional mutagenesis leading to T-cell malignancies.

The proposed studies continue the development of CARs for use in novel therapies for malignancies. Thus far, these studies have suffered from the relatively short duration of survival of the manipulated T cells *in vivo* and thus only modest measurable therapeutic results. The proposed study will make use of new CARs aimed at improving T-cell signaling and survival. The investigators' use of SB transposition provides a relatively low-cost approach to improving integration efficiency of DNA plasmids, compared with clinical-grade recombinant retro- and lentiviruses, and it also links state-of-the-art cell culture technology to these evolving attempts at immunomodulation. Time

will tell whether the 10 years of optimistic predictions of the utility of SB are matched by the performance of this system in the clinical setting.

#### ACKNOWLEDGMENTS

The author is a member of the NIH RAC, but the opinions expressed in this editorial are his own and do not necessarily represent the opinion of this committee.

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